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LABORATORY AND FIELD ASSAY OF ARSENICAL DIPPING FLUIDS.[1]

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INTRODUCTION.

The use of arsenical dipping fluids for the treatment of cattle infested with Texas-fever ticks is increasing. A mixture termed by the Bureau of Animal Industry "standard arsenical solution" is prepared from white arsenic, sal soda, and pine tar, and is largely used for both official and private dipping operations. Proprietary dipping fluids also have appeared on the market to some extent. Previous publications [2] of the bureau contain directions for the preparation of "standard arsenical solution," together with general information of importance to users of arsenical dips.

During the last few years wide practical experience of the bureau with all kinds of arsenical dips in the field has shown with increasing forcefulness that one of the greatest obstacles to the successful use of these preparations, and consequently to the effective prosecution of the tick-eradication work now progressing so well over considerable areas, lies in the uncertainty attached in many cases to the composition of these dips. There is no doubt that arsenical baths, properly prepared and used, are very effective tickicides and cause little injury to cattle. But the Texas-fever tick is a resistant organism. Destruction only follows its immersion in rather strong solutions of arsenious oxid, so strong in fact that if made only a little stronger the cattle themselves will begin to show effects. That is, the margin of safety within which solutions of this violent poison may be satisfactorily used is rather narrow. Too little fails to kill the ticks; too much injures the cattle. In either case the cause of tick eradication

[1] A popular account of laboratory tests for actual arsenious oxid and for total arsenic, together with methods of field assay for dips used in tick eradication. Of special application to officials and others concerned with the analysis and control of these preparations.

[2] Farmers' Bulletin 498; Bureau Animal Industry Circular 207.

receives a setback, not only through the wasted labor of ineffective dippings or the economic loss of injured cattle, but still more through the arousing of distrust or even enmity in those very persons whose willing cooperation in tick-eradication work is most to be desired.

The causes which may lead to the use of a bath of the wrong strength are rather numerous. Impure materials may be purchased; mistakes in measurements or computations are sometimes made even by a careful man. But these things can all be checked and guarded against; the greatest difficulties arise in maintaining the bath at the right strength, once it has been prepared. A fresh bath can not be made up every time a few cattle are to be dipped. Practical considerations render it necessary to use the bath over and over again, perhaps during a period of several months, sufficient fresh fluid being added from time to time to replace that carried out by the cattle. During such a period of time, especially in the hot summer weather, evaporation of water from the dip naturally tends to concentrate it. This may be compensated for in a measure by marking the level of the dip on the side of the vat before a period of disuse, and then filling up to the mark with water when the dip is used again. But it is difficult to construct a vat holding one to three thousand gallons so as to be entirely free from leaks. Therefore it is uncertain in any case how much of the lowering of the level of the dip is attributable to evaporation and how much to leakage. Leakage may likewise be in as well as out; that is, rain, surface water, or ground water may enter the vat.

Even if these difficulties are avoided, there is still another factor which evades all precautions, the fact that used arsenical dipping fluids may in course of time undergo a process of oxidation whereby the arsenious oxid originally present as sodium arsenite is converted to arsenic acid or sodium arsenate. Various observers have noted such a tendency, but have usually attached little importance thereto, assuming it to be caused by a slow and relatively insignificant chemical reaction. It has remained for Fuller,[1] working in this laboratory, to show that the change is essentially caused by the growth of microorganisms in the fluid—that is, it is a biological process and not a simple chemical action—and furthermore that it may become very extensive, converting nearly all the arsenic into the oxidized form. Tests by the Zoological Division of the bureau indicate that arsenic in the form of arsenate is probably a less effective tickicide than when present as arsenite, while at the same time decidedly poisonous to animals. Laws,[2] as the result of a certain number of experiments, has concluded that arsenic existing as arse-

[1] Bureau Animal Industry Circular 182.

[2] Laws. The Tick-killing Properties of Sodium Arsenate. The Agricultural Journal of the Union of South Africa, 1913, vol. 5, p. 915.

nate is probably somewhat less than half as powerful as arsenic in the form of arsenite in its effects upon both cattle and ticks. Also it has recently been observed that arsenical baths under certain conditions sometimes display the converse phenomenon of reduction—that is, arsenate tends to be reconverted to arsenite. Laws, in the article above mentioned, suggests that the phenomenon of reduction may likewise be attributable to the action of microorganisms, of course of different species from the organisms which cause oxidation. Recent work in this laboratory has substantiated the correctness of Laws's surmise. Pending the completion of certain researches it may simply be stated here that both phenomena have been observed to occur in baths in actual use in the field, sometimes in the same bath; that is, a single bath may show alternating tendencies in the two directions, first toward oxidation, then toward reduction followed by oxidation again, and so on. The primary condition which determines in which direction the change shall progress at any given time or in any particular bath is the degree of use which the bath is receiving. Under present ordinary conditions which appear to prevail in the field a gradual oxidation may be looked for. It is only in vats through which cattle are passed in exceptionally large numbers and at very frequent intervals, such as the vats at some of the stockyards centers, that reduction as a separate phenomenon becomes apparent.

A consideration of the above facts renders sufficiently obvious the necessity for some analytical control of the baths used for dipping.

The assay of arsenical dipping fluids, at least with sufficient accuracy for practical purposes, is not a difficult matter. It is nowhere described in chemical literature, however, and the average chemist, when offered the problem, will be somewhat daunted, not knowing how he may best set to work to obtain good results without a considerable expenditure of labor. It is believed that some of the methods herein to be described can be successfully executed by persons who possess but a limited chemical training. In almost every section of the country there should be some one, pharmacist, physician, veterinarian, instructor in school or college, or even student, who would find it worth while for a comparatively small fee (provided a sufficient number of samples are sent in from various sources) to undertake the assay of such preparations. A 4-ounce sample[1] is sufficient, though a larger quantity is rather more convenient for the analyst.

It is also purposed to describe a portable testing outfit that has been devised for the use of bureau inspectors in the field, and that enables them, without the possession of any chemical knowledge whatever, at the side of the vat and in a few minutes, to determine

[1] For precautions necessary in sending samples see page 4.

the strength of arsenical solutions prepared after the "standard formula."

It is necessary therefore to describe (1) methods for the determination of actual arsenious oxid, and (2) methods for the determination of "total arsenic," that is, methods which shall include arsenic present in the oxidized form as well as that existing as actual arsenious oxid. It is necessary also to describe different variations or modifications of processes for these two determinations, respectively appropriate for use by (1) trained chemists with abundant laboratory facilities, (2) persons possessing but slight chemical training and equipment, and (3) persons in the field possessing no chemical knowledge or training whatever, who obtain their results by manipulation of an "outfit" prepared by a trained chemist.

LABORATORY METHODS.

It is to be distinctly understood that the methods here described are not in all cases adapted or intended to reach a high degree of accuracy from a chemist's viewpoint, though as a matter of fact most of them are very accurate under especially favorable conditions. A certain variation must necessarily be allowed in the composition of baths prepared in the field. Again, experience has shown that the percentage of arsenic in such baths may vary within certain limits without perceptible effect upon either their effectiveness or their safety. Therefore the purpose of assay is not primarily to determine the exact percentage of arsenic—though the nearer this result is approached the better—but to insure that the composition of the baths used shall fall within certain limits. Extreme accuracy, which is not necessary, must be sacrificed to simplicity and convenience, which are necesssary to permit the execution of very frequent tests.

METHODS FOR ACTUAL ARSENIOUS OXID.

Whatever method may be employed for the determination of actual arsenious oxid, there are certain precautions connected with the taking and the storage of samples which can not be omitted if the results are to be of any value whatever. As already noted, the arsenic in used arsenical baths tends to change its degree of oxidation, mainly through the action of microorganisms. Other things being equal, the rapidity with which this change takes place is greatly influenced by temperature, so much so that a sample contained in an ordinary cork-stoppered bottle and exposed to summer heat may show more change after one day than it would show after a week in the original vat in its comparatively cool location underground. The rate of the change can accordingly be retarded by keeping the sample cold, as by storage in an ice box after receipt; but if the sample has trav-

eled any considerable distance the mischief probably has already been done before its receipt. The growth of microorganisms, and consequently the change in degree of oxidation, can be inhibited by the addition of an appropriate antiseptic, such as formaldehyde, carefully added from a medicine dropper, in the proportion of 5 drops of the commercial 37 per cent solution to each 100 c. c. of bath. Carbolic acid and mercuric chlorid are wholly inappropriate for the purpose. Also since oxidation obviously can not take place in the absence of air, it is best to fill the bottle nearly full with the sample, leaving only a little air space, cork, and cover the cork and lip of bottle (which must be dry) completely with melted sealing wax, paraffin, rosin, or some similar material. Whatever means are employed to preserve the sample the operations ought to be executed at the vat side immediately after taking the sample. A few matches will furnish the heat necessary for melting the sealing material.

No sample should, of course, be taken from a vat until the contents of the latter have been thoroughly stirred up. Aside from general reasons for this practice in all sampling it is entirely possible that the oxidation of an arsenical bath standing at rest in the vat may proceed from the surface downward. That is, the upper few inches in free contact with air might have become almost entirely oxidized while toward the bottom the bath might be still in its original condition, or might even be undergoing reduction.

The first step for the analyst, therefore, is to determine whether the sample has been properly taken and properly preserved during shipment. If the necessary preacutions have not been observed, the analyst is justified in reporting only a "probable" or "provisional" figure for "actual arsenious oxid."

The final measurement of arsenic is made in all cases by titration with standard iodin solution and starch indicator, much used by chemists for a variety of purposes. The necessary solutions are the following:

(1) *Starch solution.*—Stir up about a gram of starch, best obtained from the druggist, in about 20 c. c. of water and add the mixture slowly to about 200 c. c. of boiling water. Continue gentle boiling about 5 minutes. The solution should be freshly prepared every day.

(2) *Diluted sulphuric acid (10 per cent).*—Pour 100 grams (or 55 c. c.) of concentrated sulphuric acid *slowly, in a small stream and with constant stirring,* into 825 c. c. of water.

(3) *Standard solution of arsenious oxid.*—Weigh out accurately and exactly 2.5 grams of the purest obtainable white arsenic, and about 10 grams of sodium bicarbonate, bring into a capacious beaker or flask with about 200 c. c. of water, and boil gently until the arsenic is all dissolved. Cool, and cautiously add dilute hydrochloric or

sulphuric acid until the solution is slightly acid to litmus paper or methyl orange, or, best of all, phenolphthalein. When thoroughly cooled to room temperature, make up the solution to exactly 1,000 c. c. Each cubic centimeter will then contain 0.0025 gram arsenious oxid. The chemist[1] will recognize this as approximately a "twentieth-normal" solution. If well stoppered, the solution will keep a year or more.

(4) *Standard iodin solution.*—Weigh out about 6.5 grams of iodin and 20 grams of potassium iodid. Cover with about 100 c. c. of water and leave, occasionally mixing, until all iodin is dissolved. Then make up to 1 liter. To obtain the exact strength of the iodin solution, measure exactly 25 c. c. of the standard arsenious oxid solution into a 200 c. c. flask or wide-mouth bottle, add about 25 c. c. of water, about 2 grams of sodium bicarbonate, and a few cubic centimeters of starch solution. Then, while shaking the flask, run in the solution of iodin from a burette[2] until the blue color of iodized starch just remains permanent. Twenty-five cubic centimeters of the standard arsenious oxid solution contain exactly 0.0625 gram of arsenious oxid. Therefore, the number 0.0625 divided by the number of cubic centimeters of iodin solution necessary to just produce a permanent blue color, gives a quotient which represents the exact weight of arsenious oxid to which each cubic centimeter of iodin solution is equivalent. For example, suppose 26.2 c. c. of iodin to be required. Then $0.0625 \div 26.2 = 0.00239$ gram arsenious oxid per each cubic centimeter of iodin. The standard iodin solution should be kept only in glass-stoppered bottles, as it will be weakened by contact with rubber or cork. If preserved in tightly stoppered and well-filled bottles in a cool, dark place, it retains its strength a considerable time, but in practice it should be standardized against the standard solution of arsenious oxid every week or two.

For the actual analysis two methods will be described. If the sample is a concentrated preparation, it should always be reduced by dilution with water to the strength at which it is intended for use in actual dipping.

METHOD "A" FOR ACTUAL ARSENIOUS OXID.

Measure 25 c. c. of bath into a beaker, flask, or bottle of convenient size, add about a gram of sodium bicarbonate and about 10 c. c. of starch solution. Run in standard iodin solution until the blue color appears just as in standardizing the iodin solution, though here it

[1] The experienced chemist will undoubtedly employ solutions of definite normality. The directions here given are intended, as simply as possible, to meet the needs of those who may be without much chemical knowledge or a highly accurate analytical balance, perhaps lacking even burettes and pipettes.

[2] A 25 c. c. measuring cylinder may be employed if a burette is lacking.

usually fades out in a minute. In the case of very dirty baths the color may appear simply as a violet, reddish, or brownish deepening of the naturally dark color of the bath itself. In such cases it is well to have two flasks at hand, both containing the measured portions of bath and starch solution. Then by running iodin solution into one of the flasks and constantly comparing the color with the color of the liquid in the other flask, the point at which the change occurs may be more easily distinguished. Since the color is not at all permanent in the case of old and dirty baths, and since slight changes of tint are impossible to distinguish in such baths, it is good practice to add the iodin solution in quantities of 0.5 c. c. at a time when it is suspected that the end point is close at hand, then to mix thoroughly and immediately observe the color. This quantity of iodin solution is usually sufficient to produce a very pronounced change of tint if the end point of the titration really has been reached. The final reading should then be corrected by subtracting 0.25 or 0.50 c. c., whichever is judged nearest correct. The number of cubic centimeters of iodin solution needed to produce the blue color, multiplied by the strength of each cubic centimeter in terms of arsenic, and this again by 4, will give the grams of arsenic per 100 c. c. of bath—that is, the percentage. For instance, suppose that each cubic centimeter of iodin solution was found to be equivalent to 0.00239 gram arsenious oxid, and that 18.3 c. c. of iodin solution were employed in the titration of 25 c. c. of a bath under examination. Then the bath contains $0.00239 \times 18.3 \times 4 = 0.175$ per cent of actual arsenious oxid.

Theoretically, method "A" can not be applied with perfect accuracy to dipping baths, on account of the possibility that substances other than arsenious oxid—such as tar and organic matter derived from the cattle—may absorb some iodin and thus lead to false results. Practically, however, this method has been shown by many tests to give useful results on dipping baths of all ages prepared after the "standard formula" recommended by the Bureau of Animal Industry. (See p. 1.) That is, the various ingredients of the tar and the organic matter derived from cattle actually do not interfere fatally, but absorb iodin so slowly that the end point with starch is obtainable without difficulty, although it usually fades out in a brief time. Therefore, method "A" is suggested for use in the practical testing for ordinary purposes of baths prepared after the "standard formula." It must not, however, be employed on baths prepared from any proprietary dip unless it is certainly known that the particular dip contains no substance which can interfere. Since it is the present policy of the bureau to permit for use in official dipping only such proprietary preparations as may be satisfactorily tested by means of the field outfit later to be described, and since the field test is practi-

cally identical with method "A," the latter method may be safely employed for the examination of samples known to be prepared from proprietary dips which have received such official recognition.

METHOD "B" FOR ACTUAL ARSENIOUS OXID.

Measure 25 c. c. of bath into a small beaker, add 5 c. c. of 10 per cent sulphuric acid and 0.25 gram of acid-washed blood-charcoal. Stir thoroughly and heat nearly to boiling—best on a steam bath—for five minutes. Then filter and wash with hot water until the filtrate amounts to a little over 100 c. c. If the bath appears to be very heavily loaded with dirt or tar, the addition of about a gram of acid-washed kieselguhr (infusorial, siliceous, or diatomaceous earth), followed by thorough stirring before filtration, will greatly hasten the passage of the liquid through the filter. When the filtrate has been thoroughly cooled to room temperature, sodium bicarbonate is added until 1 or 2 grams are present in excess after effervescence ceases. In case the bath was prepared from a proprietary product, dilute to about 200 c. c. before adding sodium bicarbonate, and lastly also add 2 grams potassium iodid.[1] Finally, the solution is titrated with standard iodin and the result calculated as under method "A." If the greatest possible accuracy is desired a correction must be made for loss of arsenic through the use of blood-charcoal. This substance notably adsorbs arsenious oxid, and even after thorough washing a slight amount remains unaccounted for, which presumably still is retained by the charcoal. The amount of arsenic so lost appears—at least within practical limits—to be independent of the concentration of the bath, but is naturally dependent upon the amount of charcoal employed. Hence the charcoal must be rather carefully weighed or measured, and the amount of the correction for each lot of charcoal must be established by running comparative titrations on two portions of a solution of pure arsenious oxid, one direct, the other after the treatment with charcoal, filtration, and washing, which has been described.[2] The power of the charcoal to produce a water-white filtrate in which the blue end point is sharp and permanent far outweighs its disadvantages if accurate results are desired.

Method "B," like method "A," should not be applied to proprietary dips unless they are permitted for use in official dipping or are otherwise known to be free from interfering substances.

[1] Dilution and the addition of potassium iodid aids in nullifying the interfering effect of cresylic acid and other substances capable of absorbing iodin which may be present in proprietary dips. Phenols rapidly absorb iodin from a solution containing sodium bicarbonate. The presence of free carbonic acid, which of course saturates the liquid during titration, greatly reduces the rate of absorption. Finally, if the solution contains a certain concentration of potassium iodid, and not above a certain concentration of the phenols, the end point comes out perfectly sharp, of good color and permanency, following a titration figure which appears wholly unaffected by the presence of phenols.

[2] The sample of blood-charcoal at present in use in this laboratory demands a correction of 0.2 c. c. of twentieth-normal iodin for 0.25 gram charcoal.

METHOD FOR "TOTAL ARSENIC."

Strictly speaking the oxidized form of arsenic no longer contains arsenious oxid as such (As_2O_3) but only arsenic oxid (As_2O_5). Nevertheless, for the purpose of making simple and convenient comparisons, it is necessary to refer all quantitative statements regarding arsenic compounds to the common basis of their equivalent in arsenious oxid. Therefore, the term "total arsenic" is employed to signify all arsenic present in any form of combination or degree of oxidation, expressed in terms of arsenious oxid.

The method to be described is a standard method based on the well-known fact that the reaction $As_2O_3 + 4I + 2H_2O \rightleftharpoons As_2O_5 + 4HI$ is reversible, going from left to right in neutral or alkaline solutions, and from right to left in solutions which are freely acidified with a strong mineral acid. The reaction from left to right is the basis for the previously described methods for the determination of actual arsenious oxid. To determine total arsenic it is only necessary to allow the reaction to progress to completion from right to left, then to determine the resulting arsenious oxid in a manner similar to that already described.

The solutions and reagents necessary, in addition to the same standard iodin solution, starch solution, dilute sulphuric acid, and solid sodium bicarbonate, are anhydrous powdered sodium carbonate, solid potassium iodid, a one-tenth per cent solution of methyl orange, possibly concentrated sulphuric acid, and lastly a solution of sodium thiosulphate containing about 25 grams per liter.

The first step is to clarify and decolorize the solution. Hence proceed exactly as in method "B" for actual arsenious oxid (p. 8) until the filtered solution has been obtained. When both actual and total arsenious oxid are to be determined it is most convenient to double the quantities of both acid and charcoal, and to filter into a 200 c. c. volumetric flask. After thorough washing and cooling, the contents of the flask are made to the mark, mixed, and divided into two equal portions, one for actual arsenious oxid, the other for total arsenic. In either case transfer the solution containing the equivalent of 25 c. c. of the original bath to a 200 c. c. beaker, add 4 c. c. of concentrated sulphuric acid and about 2 grams of potassium iodid, boil gently until the volume of liquid is reduced to 50 c. c., then cool to room temperature. The next step is to remove free iodin from the solution. This may be done while the solution is still in the beaker, by adding the solution of sodium thiosulphate drop by drop from a burette until the yellow color of free iodin just disappears. Any excess of thiosulphate must be carefully avoided. A safer procedure for one not experienced with the method is to wash the contents of the beaker into a flask of 600 to 800 c.c. capacity, dilute

to about 200 c. c., nearly discharge the color of iodin by sodium thiosulphate, add a little starch solution, and continue the cautious addition of thiosulphate solution until the blue color disappears. Then add standard iodin solution from a burette until the faintest possible persistent blue remains. Whichever method is used, the solution at this stage should be in a capacious flask, and should be diluted to about 300 c. c. Add a few drops of methyl orange, then render alkaline by the cautious addition with a spatula of anhydrous sodium carbonate. When the solution is clearly alkaline wash down any solid adhering to the neck or sides of the flask and add dilute sulphuric acid until the reaction is clearly acid, taking care that no particles of sodium carbonate remain undissolved. Then add a liberal excess of sodium bicarbonate, 10 c. c. more starch solution, and titrate with standard iodin in the usual manner. The final titration should of course be corrected for the adsorption of arsenious oxid by blood-charcoal. No additional correction for adsorption of arsenic oxid appears necessary. No extra addition of potassium iodid appears to be necessary in case cresylic acid, etc., is present.

The above-described methods for actual arsenious oxid and for total arsenic have proved reliable for the examination of all products thus far permitted for use in official dipping, and it is probable that they will be found applicable to any proprietary products which may receive such official recognition in the future, though obviously a positive statement on this point can not be made. The experienced chemist will naturally think of other methods which might be applied. For example, the acidified and filtered bath may be treated in a separating funnel with ether or some other appropriate organic solvent to extract cresylic acid, fatty acids, etc.; total arsenic may be determined after the destruction of organic matter by appropriate digestion or incineration, or it may be separated by distillation; while it is known that some chemists prefer to determine directly arsenic existing as arsenic acid by titration with uranium acetate.

FIELD METHODS OF ASSAY.

A field method of assay is generally based upon a laboratory method; apparatus, reagents, etc., being simplified to the extreme in the interests of durability and portability, and the operations being reduced to the fewest and simplest, that they may be successfully executed by persons wholly unacquainted with chemistry as a science. But a skilled chemist in the laboratory to supervise the field tests is even more necessary than if all the work were actually performed in the laboratory itself, for upon his experience and care reliance must be placed to standardize the methods in such a way that useful data may actually result from the manipulation of inadequate apparatus by unskilled hands. Of course, it is only excep-

tionally that a laboratory method can be modified so that it is of any practical use whatever in the field, and in any event it is almost certain to lose something in accuracy. One thing is absolutely essential, that the field operator shall follow the instructions given him to the minutest detail, no matter how irrelevant or unimportant they may appear to him.

FIELD METHOD FOR ACTUAL ARSENIOUS OXID.

The field method at present employed by the bureau for actual arsenious oxid is simply an adaptation of laboratory method "A" for the same substance. The outfit is pictured in figure 1, and each part composing it will be described in detail.

(1) *The case.*—The carrying case for the outfit is a rectangular box with a hinged cover, made of five-sixteenths-inch oak, of inside dimensions $7\frac{1}{2}$ by $5\frac{1}{8}$ by $1\frac{7}{8}$ inches. The interior partitions, of thinner and softer wood, are sufficiently indicated in the diagram. The case must be strongly mortised or nailed together, not simply glued, and should be varnished inside and out.

(2) *The utensils.*—Bottle A′, fitting into compartment A of the case, is an ordinary 3-ounce wide-mouth bottle of clear glass.

Measuring cylinder C′, fitting in compartment C, is of ordinary type, of 25 c. c. size, graduated to half cubic centimeters. Preferably the figures indicating the graduations read down only. C″ is a bristle swab for cleaning. It will be noted that the partitions of compartment C are cut away at the bottom to admit the foot of the cylinder. At the point *p* on the back wall of the case is fastened a quarter-inch pad of cork to protect the cylinder from breakage. The swab C″ is put into compartment C after the cylinder, thus protecting the latter from breakage on that side.

(3) *The reagents.*—The iodin solution, or, as it is termed for field use, the "test fluid," is contained in bottle D′, a 4-ounce standard-shaped "sample oil" bottle, preferably of amber glass and provided with a "flat-hood" glass stopper. A half-inch ring of the $1\frac{3}{4}$-inch (measured flat) thin rubber tubing, manufactured for use with Gooch crucibles, is drawn over the shoulder of the bottle, cemented in place, and coated with collodion. At the bottom of compartment D is fastened by a screw a conical spiral spring, the upper whorl of which is large enough to inclose the bottom of the bottle. In placing the latter in the case the bottom is inserted in the whorl of the spring and the bottle then pressed down until it slides into place. The partition between C and D is cut away in a semicircle at the point *q* to allow the fingers easy access to the bottle when it is to be removed.

The test fluid is of such strength that in the actual performance of the test each cubic centimeter of it employed represents exactly one

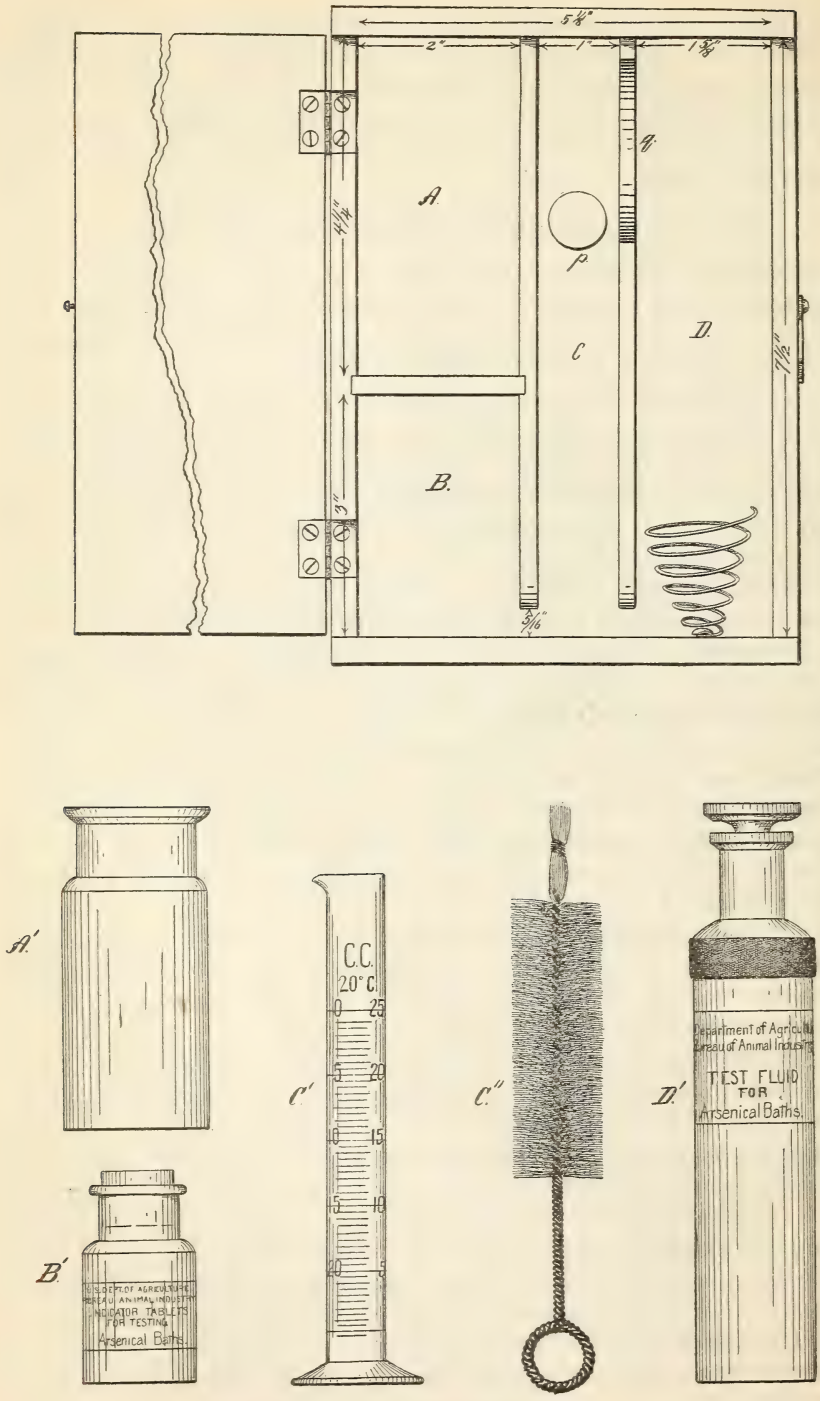

FIG. 1.—Test outfit for arsenic baths.

one-hundredth of 1 per cent of arsenious oxid in the bath under test. Therefore its strength should be originally fixed by standardizing it with the field apparatus against an average sample of used bath from the field in which the percentage (adjusted if necessary by the addition of a little concentrated solution of arsenious oxid in sodium carbonate) of actual arsenious oxid is close to 0.20 per cent and is accurately known through laboratory analysis. The true strength of this empirically standardized iodin solution should then be ascertained by titration against a strictly tenth-normal or twentieth-normal solution of arsenious oxid, and the result obtained may thereafter serve as the basis for the preparation of subsequent lots of test fluid. Obviously the true strength of the iodin solution will be influenced to some extent by the method of graduation of the cylinder, whether graduated "to contain" or "to deliver," and also by the depth of the meniscus, which in turn is influenced by the diameter of the cylinder. Hence, all field outfits under the supervision of a single laboratory should be fitted with cylinders of uniform model. Reserve supplies of test fluid should be kept in small, well-filled, tightly closed glass-stoppered bottles, and in a cool, dark place.

In addition to the test fluid, starch and sodium bicarbonate, or some equivalent substance, are of course necessary. In fact, the practical preparation of a satisfactory form of starch has been the greatest difficulty attached to the whole process, though at the same time the key to its success.

It has been known for many years that by the use of alcohol starch may be precipitated in water-soluble form, also that high-percentage, yet mobile, starch solutions may be obtained through proper treatment with hydrochloric acid, but the working out of a practical process for the preparation in quantity of a dry starch readily soluble in cold water and appropriate for use as an indicator appears not to be recorded.

Into a 5-liter round flask with a long neck is brought 400 grams potato starch, 2,300 c. c. distilled water, and, lastly, 80 c. c. of normal hydrochloric acid. The flask is well shaken to thoroughly wet and distribute the starch and is floated in a kettle of water previously brought to vigorous boiling. The neck of the flask conveniently rests on the side of the kettle at an angle of about 45°, and as soon as the flask is brought into the bath it is gently but continuously rotated about its longitudinal axis. As the flask becomes hot the starch forms an evenly distributed, uniform jelly, which in about 7 minutes from the time of starting begins to liquefy and to fall away from the wall of the flask. When this stage is reached the mouth of the flask is loosely closed with an inverted beaker and the flask left in the boiling bath with an occasional rotation until the liquid becomes mobile

and shows no lumps of gelatinized starch remaining, which should take a little over an hour. The flask is quickly cooled in running water until it can be comfortably handled, then a few drops of methyl orange are added, followed by concentrated ammonia to alkaline reaction. Next is added 800 c. c. of 95 per cent alcohol with thorough mixing, and after a few minutes standing to allow air bubbles to separate, the liquid is strained through moderately coarse muslin. The addition of this amount of alcohol is insufficient to permanently precipitate any starch, but notably thins out the original aqueous solution. Starch will separate some time after the solution has become cold, but with proper management ample time remains for the subsequent necessary operations. The solution, still at 40° to 45° C., is run through a number of fine jets into 4,000 c. c. of 95 per cent alcohol, under continuous stirring. The whole is left for at least 48 hours with an occasional thorough stirring, after which most of the supernatant alcohol is decanted, and the rest used to transfer the starch to a 2-quart narrow percolator provided with a filter plate which is covered with filter paper or cloth. Here it is percolated with 95 per cent alcohol, being stirred up with a stick at intervals to prevent the formation of clumps or fissures, until the alcohol comes through of a specific gravity indicating a strength of 90 per cent. The starch is then transferred to a Büchner funnel, well drained with suction, and then spread out to dry in a moderately warm place. The starch so prepared is a fine white powder, more or less compacted to friable lumps, which completely disintegrate under slight pressure. A little of it thrown into cold water in less than a minute dissolves sufficiently to yield a good blue upon the addition of iodin and potassium iodid. Moistened with water or diluted alcohol it becomes gummy and dries out to a horny mass, difficultly soluble in cold water. The efficiency of the preparation therefore is dependent upon its fine state of subdivision, and care must be taken during the process not to expose it to air until after thorough digestion with alcohol of 90 per cent strength. It should be passed through a 60 or 80 mesh sieve and protected from moist air.

The soluble starch may be thoroughly mixed with 10 times its weight of powdered sodium bicarbonate and the mixture divided into powders of about 0.6 gram, which may be packed in a pasteboard box fitting into compartment B of the case. On the large scale, however, it is much better to make the mixture up into tablets,[1] after the following formula:

[1] It is something of an art to make good tablets. No one should attempt it until thoroughly conversant with the principles of the process, and then only on a small scale until experience is gained.

Milk sugar in fine powder	grams..	100
Sodium bicarbonate, in powder	do....	1,000

Mix, moisten with dilute alcohol, granulate, dry thoroughly at only slightly elevated temperature, and grind granules to pass a 20-mesh sieve.

Mix granules with—

Soluble starch	grams..	100
Raw potato starch	do....	75
Talcum, U. S. P	do....	25

Compress into tablets of 0.65 gram.

The practical tablet maker will be tempted to criticize this formula because it carries so much powder; but since the soluble starch is ruined by wetting it can not be incorporated into the granules, so that there appears to be no escape from this drawback, which at any rate has not prevented the practical preparation of the tablets on a large scale for the use of the bureau.

The tablets are put up in the 1-ounce wide-mouth bottle B′, fitting into compartment B of the case.

On the inside of the cover of the case is glued a printed instruction sheet, which is protected by pyroxylin varnish. The instructions read as follows:

UNITED STATES DEPARTMENT OF AGRICULTURE,
BUREAU OF ANIMAL INDUSTRY.

TEST CASE FOR ARSENICAL BATHS.

Not to be used on baths prepared from proprietary preparations except on special instruction.

Keep test fluid in a cool dark place in glass-stoppered bottles only.

DIRECTIONS.

1. Fill clean graduate with bath, setting the *top* edge of the surface of the bath on the upper graduation (zero or 25 c. c.), pour (draining out drops) into clean wide-mouth bottle, add one white indicator tablet, and gently swirl or shake till tablet is nearly all dissolved.

2. Rinse graduate with clean water, shake out adhering drops (or rinse with a little test fluid), and fill to upper graduation (zero) with test fluid, setting the *bottom* of the curved surface on the mark.

3. While gently swirling bottle, slowly pour in test fluid from the graduate until the blue or violet color remains permanent for a half minute throughout the entire contents of the bottle after thorough mixing. Avoid excess of test fluid, adding only a few drops at a time toward the end.

The number of cubic centimeters (reading at the bottom of the curved surface) of test fluid added to just produce the color gives the number of hundredths of 1 per cent of arsenious oxid in the bath.

If the assay is not performed at the vat directly after taking the sample, the same precautions to prevent changes in degree of oxidation must be observed as described under "Laboratory Methods for Actual Arsenious Oxid" (p. 4). The scope of application of the method is of course subject to exactly the same limits as laboratory method "A" for actual arsenious oxid (p. 6).

FIELD METHOD FOR "TOTAL ARSENIC."

Since, pending further investigation, it is not possible to credit arsenic in the higher form of oxidation (arsenic acid or arsenates) with definite value as a tickicide, the efficiency of dipping baths must be judged for the present solely on the results afforded by the test for actual arsenious oxid. On the other hand, studies of changes in the composition of a number of dipping baths in actual service in the field, together with practical experience, have so far failed to show a clearly defined danger that the average bath, in which the strength of actual arsenious oxid is maintained, is likely to reach a degree of oxidation which may render it dangerous to cattle. Nevertheless, in view of obvious possibilities in these two directions, it is desirable to possess a field method for the estimation of total arsenic. The method to be described makes use of a chemical reaction recently discovered by the writer,[1] namely, the reduction of arsenic acid to arsenious acid by thiosulphuric acid. After reduction and removal of excess thiosulphuric acid by iodin in acid solution, sodium bicarbonate may be added and the arsenious oxid, now representing the total arsenic, may be titrated in the usual way. The method is necessarily more complicated, more tedious, and less accurate than the simple method for actual arsenious oxid. Nevertheless, rather comprehensive tests by representatives of the bureau in the field indicate that, if conditions demand such a test and no better can be discovered, the one at hand will afford useful results.

In addition to the outfit and supplies already described, the following supplies are necessary:

1. *"Red tablets":*

Talcum powder, U. S. P.	grams..	10
Raw potato starch	do....	30

Mix, and stir in 0.1 gram Sudan Red III dissolved in sufficient ether to distribute the color, and evaporate off ether at a moderate temperature with frequent stirring.

Add:

Sodium bicarbonate in fine powder	grams..	3
Special soluble starch	do....	10
Mix, and add potassium pyrosulphate powdered to pass a 40-mesh sieve	do....	125

Mix well and compress into tablets of such size that 1 tablet will neutralize 9 to 10 c. c. of normal alkali. The mixture, not being granulated, can not be run through the hopper of a power tablet machine, but must be fed by hand into the die from the table of the machine.

2. *"Blue tablets":*

Ultramarine blue	grams..	1
Talcum powder, U. S. P.	do....	5
Sodium thiosulphate, crystallized, ground to pass 20-mesh sieve, and air-dried	do....	100

Mix and compress into tablets of such size that 1 tablet will be equivalent to about 22 c. c. of twentieth-normal iodin.[2]

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[1] Journal of Agricultural Research, 1914, vol. I, p. 515.

[2] It is probable that potassium iodid should also be added in making up these tablets in order to prevent some oxidation of arsenious oxid by iodin in acid solution in case "oxidized arsenic" is originally present in amount sufficient to nearly completely use up the thiosulphate.

The directions for executing the test read as follows:

1. Use regular outfit. Measure 25 c. c. of bath into wide-mouth bottle and add 1 blue tablet. When entirely dissolved, add 1 red tablet, and after this has entirely fallen to powder gently but continuously swirl the bottle for 3 minutes, then let it stand for 7 minutes more with an occasional mixing.

2. Next add test fluid from the graduate until the blue color just remains permanent for at least 10 seconds throughout the whole of the well-mixed liquid. Add but a few drops at a time toward the end and avoid excess, but pay no attention to the actual volume of test fluid added at this stage.

3. Now add *two* white indicator tablets and agitate till mostly dissolved. Fill the graduate to upper mark (zero) with test fluid, adding it just as in the estimation of "actual arsenious oxid" until the blue color just reappears permanently, avoiding excess.

The number of cubic centimeters of test fluid thus added to bring back the blue color represents hundredths of 1 per cent of "total arsenic" in the bath.

If "oxidized arsenic" is present in only very small amount the results are likely to be somewhat low, owing to some formation of arsenious sulphid, but in any event such small amounts of "oxidized arsenic" are without significance. Again it is obvious that the quantity of thiosulphate used will not show the presence of "oxidized arsenic" in amount much over 0.2 per cent of the bath, but this appears amply sufficient, for if so much is present, in addition to the actual arsenious oxid, which of course is determinable in practically any amount, the bath certainly should not be used for dipping.

THE INTERPRETATION OF RESULTS.

The "standard arsenical solution" is recommended by the bureau for use in two strengths—first, 10 pounds of white arsenic and 25 pounds of sal soda to each 500 gallons, and, second, 8 pounds of white arsenic and 24 pounds of sal soda to each 500 gallons of bath. The theoretical percentages of arsenious oxid will therefore be practically 24 hundredths of 1 per cent for the 10–25 formula, and 19 hundredths of 1 per cent for the 8–24 formula. Practical experience, however, has shown that the baths work with perfect satisfaction so long as the percentages of "actual arsenious oxid" do not drop below the following limits: For the 10–25 formula, 22 hundredths of 1 per cent; for the 8–24 formula, 175 thousandths of 1 per cent. These figures therefore represent minimum percentages below which the contents of the respective baths in "actual arsenious oxid" should not be allowed to fall. The maximum allowed percentages of "actual arsenious oxid" should be but little above the theoretical figures; say, 25 hundredths of 1 per cent for the 10–25 formula and 20 hundredths of 1 per cent for the 8–24 formula.

Respecting the maximum allowable percentages of "total arsenic," it is not yet possible to make any statements based upon positive experimental evidence. One might tentatively set the following limits which experience has indicated to be within the margin of safety: 30 hundredths of 1 per cent for the 10–25 formula; 25 hundredths of 1 per cent for the 8–24 formula.

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